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(54) Title: IMPROVED PROCESS FOR PHOSPHORYLATION AND COMPOUNDS PRODUCED BY THIS PROCESS

(57) Abstract

A process for phosphorylating primary fatty alcohols, secondary alcohols, or aromatic alcohols comprising the following steps: (a) forming an intimate mixture of one or more of the above alcohols and P_4O_{10} or partly hydrated P_4O_{10} or a mixture thereof at a temperature below 80 °C; and (b) allowing the intimate mixture to continue to react for a period of time at a temperature below 80 °C until the formation of the dihydrogen form of the phosphorylated alcohol is substantially formed.

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Improved Process For Phosphorylation And Compounds Produced By This Process

Field of the invention

The invention relates to an improved process for phosphorylation of organic hydroxyl groups and the compounds produced using this process.

Background of the invention

Whilst the following discussion highlights the invention with respect to dietary supplements, it is believed that the same principles apply to other compounds containing organic hydroxyl groups such as pharmaceutical compounds with hydroxyl groups.

The use of dietary supplements is well known, for example hormones, phytosterols or chromans. One of the problems encountered with such supplements for human ingestion is that many of the supplements are relatively water insoluble but the human digestive tract is a substantially aqueous system. Previous attempts to overcome this problem include using emulsifiers to enable an oil-based solution of the supplement to combine with an aqueous system and thus maintain the supplement's bioavailability. Consequently, it would be useful to be able to convert these dietary supplements to water soluble compounds without disturbing their inherent structure. Phosphate salts with either potassium or sodium are already found in biological tissue. Therefore phosphate salts should be tolerated by the body.

There is a diverse art for the production of organic phosphates, however none of these methods are considered to be suitable for production of complex phosphate compounds because they are either unsuitable for use on a commercial scale or there are side reactions which produce undesired by-products.

Ordinarily, phosphorylation reagents and methods are chosen to avoid significant degradation of the compound being phosphorylated. Where gentle conditions are required, then reagents such as 2:2:2–trichloroethyl dichlorophosphate, di-imidazolide chlorophosphate and di-analide chlorophosphate have been employed but have limited yields which are inadequate for commercial processes. When more severe conditions are feasible, then phosphorous oxychloride has been used, but the reaction produces a variety of by-products together with hydrogen chloride. There are other problems associated with

the fact that phosphorous oxychloride is difficult to manage which make this reagent unsuitable for use on a commercial scale.

Although P_4O_{10} [which is often incorrectly called phosphorous pentoxide] has been used for phosphorylation of ethanol and other short chain primary alcohols, it has not been used for higher alcohols and complex molecules because the temperatures used are too high and there is considerable degradation. Another reason why P_4O_{10} is not used for higher alcohols and complex molecules is that at the higher temperatures used in known P_4O_{10} processes, there is formation of a significant amount of by-products. Even with ethanol, there is a significant amount of diethylphosphate as well as monoalkylphosphate which is produced and these substances must be removed. Commercial processes use P_4O_{10} with ethanol but there is a complicated clean-up procedure because the reaction occurs at a high temperature.

Further, with secondary or tertiary alcohols P_4O_{10} causes dehydration and formation of a double bond. This dehydration is further promoted by the high temperatures at which this reaction takes place. In fact, this is a standard reagent and method for forming a double bond. This reaction has thus been considered to be unsuitable for production of complex phosphate compounds.

It is the need for lower temperatures which has led to the use of $POCl_3$ because, in the presence of a base, a lower temperature can be used and degradation is avoided. $POCl_3$ is the preferred method for phosphorylating complex molecules.

There is, therefore, a need for a reliable process for phosphorylating complex compounds so that these compounds can be used in aqueous environments.

Summary of the invention

It has surprisingly been found that P_4O_{10} can be used to phosphorylate primary fatty alcohols, secondary alcohols (including cyclohexanols) and aromatic alcohols (including phenols and chromanols). In this description and in the claims, the term "complex alcohols" refers to primary fatty alcohols, secondary alcohols and aromatic alcohols. Further, similar results may be found with partly hydrated P_4O_{10} which is commonly called polyphosphoric acid.

The complex alcohols include hormones, phytosterols, tocopherols (chromans), vitamin K1 and other oil-soluble vitamins and dietary supplements as well as pharmaceutical compounds such as Amoxycillin.

In this description, the word "intimate" is used to signify its technical meaning as known to persons skilled in the art. That is, to signify that two substances are in very close physical contact dispersed as particles which are as small as possible so that a reaction is initiated. There must be as large a surface area as possible for the reaction to initiate and this is also advantageous for further reaction.

Accordingly, there is provided a process for phosphorylating complex alcohols comprising the following steps:

(a) forming an intimate mixture of one or more complex alcohols and P_4O_{10} or partly hydrated P_4O_{10} or a mixture thereof at a temperature below 80°C; and

(b) allowing the intimate mixture to continue to react for a period of time at a temperature below 80°C until the formation of the dihydrogen form of the phosphorylated complex alcohol is substantially completed.

It is understood that in steps (a) and (b), the temperature is sufficient to ensure there is minimum degradation of the complex alcohols but the reaction will still proceed to a satisfactory extent.

The complex alcohols must either be in a liquid phase or in solution for the reaction to proceed. If the complex alcohols are not liquid at the desired temperature of reaction, the complex alcohols will need to be dissolved in a solvent in which P_4O_{10} is also soluble.

Preferably, where minimum degradation is desired, the temperature at which the reaction is performed is in the range from 0 to 50°C. More preferably, the temperature is in the range from 0 to 40°C.

Preferably where the period of time in step (b) is minimized, the temperature at which the reaction is performed is about 70°C.

The ratio of P_4O_{10} to complex alcohols will depend on the temperature at which the reaction occurs. At the higher temperatures, the ratio of phosphorus to complex alcohols is substantially equimolar. That is, at the higher temperatures there is more efficient

consumption of the phosphate groups. At the lower temperatures, the ratio of P_4O_{10} to complex alcohols is substantially equimolar.

The period of time in step (b) is dependent on the temperature at the ratio of reagents. Where there is equimolar phosphorus, preferably the period of time does not exceed about 30 minutes. Where there is equimolar P_4O_{10}, preferably the period of time does not exceed about 10 minutes.

The choice of temperature at which the reaction occurs is dependent on the expense of the complex alcohols. For example, Amoxycillin is expensive therefore it is preferable to minimize the degradation of Amoxycillin.

Where lower temperatures are used and there are unreacted reagents, the unreacted reagents can be recycled. For example, if the temperature is between 0 to 40°C, the process would further comprise a step where the unreacted reagents were mixed with more P_4O_{10} and complex alcohol and steps (a) and (b) repeated.

The phosphorylated complex alcohols may be recovered as either the acid or as a salt (usually potassium or sodium) using methods known to those skilled in the art. For example, the reaction mixture from step (b) may be neutralized with potassium or sodium hydroxide then the water evaporated to recover the salt.

The pressure is typically at atmospheric because there is no advantage using higher pressures at these temperatures.

The intimate mixture is formed using methods known to those skilled in the art. Vigorous stirring is typically necessary to achieve an intimate mixture. In a laboratory, a mortar and pestle can be used. In an industrial plant, a high shear mixer would be used.

According to a preferred embodiment, formation of the intimate mixture in step (a) is performed in the presence of an aliphatic carboxylic acid excluding formic and acetic acid. In this description and in the claims, the term "aliphatic acid" refers to any aliphatic carboxylic acid except for formic acid and acetic acid. Preferably, the aliphatic acid is a free fatty acid. Examples include oleic acid and stearic acid. The aliphatic acid acts as a catalyst for the reaction and reduces the side reactions. Preferably, where a solvent is used to dissolve the complex alcohols, the solvent is a free fatty acid.

According to another form of the invention, there is provided a phosphate derivative of a complex alcohol which was produced by the above process.

Examples

The invention will now be further explained and illustrated by reference to the following non-limiting examples.

Example 1

P_4O_{10} (0.28 g) was added to 1-dodecanol (0.18 g) and stearic acid (0.02g). The mixture was stirred vigorously for 5 mins at 20-25°C. The product was analyzed by electrospray mass spectrometry which showed the formation of 1-dodecanol phosphate.

Example 2

A phytosterol extract containing mainly beta sitosterol, stimasterol and campastenol (0.4g) was mixed with polyphosphoric acid (0.8g) at 20-25°C by grinding in a mortar and pestle for 0.5 hours then let stand for 12 hours at ambient temperature. The product was diluted with acetonitrile and then analyzed by spray mass spectrometry which showed that the mono-phosphates of the sterols were present.

Example 3

17 beta-estradiol (0.27g) was mixed with polyphosphoric acid (0.3g) at 20-25°C in a mortar and pestle for 0.5 hours then let stand for 12 hours at ambient temperature. The product was diluted with acetonitrile and analyzed by spray mass spectrometry which showed that 17 beta-estradiol monophosphate had been formed.

Example 4

Alpha-phylloquinone (or vitamin K1) (0.45g in 5g oleic acid) was mixed with P_4O_{10} at 20-25°C in a mortar and pestle for 0.5 hours then let stand for 12 hours at ambient temperature. The product was analyzed which showed that the mono-phosphate was formed.

Example 5

P_4O_{10} (165.1g) was added to tocopherol (1 kg) and stirred together for 30 minutes at 70°C. The mixture discoloured to give a brown/black material which became very viscous. The material was then mixed vigorously with a mechanical stirrer for 30 minutes in water (10 l) to form a slurry. The slurry was then centrifuged, the water discarded, and the pellet collected. The pellet was then dissolved in AR ethanol (10 l). Then sodium (160.4 g) was added slowly to the solution and stirred by a magnetic stirrer. The mixture was then filtered, resuspended in AR ethanol (10 l) and heated to reflux, so dissolving the unreacted tocopherol and fatty acid. The hot dispersion was cooled and filtered to recover di-sodium tocopherol phosphate.

Example 6

P_4O_{10} (3.0g)was added to a mixture of dopamine hydrochloride (2.0g) and stearic acid (0.04g), then mixed together. To the resulting heterogenous solid was added water (0.3-0.5 ml), causing an exothermic reaction (~50°C). The resulting slurry was stirred for 2–3 minutes, then water (50 ml) was added completely dissolving the mixture. The mixture was analyzed using electro-spray mass spectrometry to find dopamine phosphate and inorganic phosphates.

Example 7

The above procedure (example 6) was repeated with amoxicillin.trihydrate (2g), stearic acid (0.04g) and P_4O_{10} (1.4g). The product mixture contained Amoxicillin phosphate and inorganic phosphates.

Example 8

The above procedure (example 6) was repeated with cholesterol (2.0g), stearic acid (0.04g) and P_4O_{10} (1.5g). The reaction mixture was dispersed into water (50 ml) then centrifuged to recover the cholesterol phase. This phase was analyzed and was found to contain unreacted cholesterol and cholesterol phosphate.

The novel process for phosphorylation has been successfully used for a variety of useful compounds and would be understood by those skilled in this art to have an obviously wider application.

The word 'comprising' and forms of the word 'comprising' as used in this description and in the claims does not limit the invention claimed to exclude any variants or additions.

Modifications and improvements to the invention will be readily apparent to those skilled in the art. Such modifications and improvements are intended to be within the scope of this invention.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A process for phosphorylating complex alcohols comprising the following steps:

   (a) forming an intimate mixture of one or more complex alcohols and P_4O_{10} or partly hydrated P_4O_{10} or a mixture thereof at a temperature below 80°C; and

   (b) allowing the intimate mixture to continue to react for a period of time at a temperature below 80°C until the formation of the dihydrogen form of the phosphorylated complex alcohol is substantially completed.

2. A process according to claim 1 wherein the temperature in steps (a) and (b) is in the range from 0 to 50°C.

3. A process according to claim 2 wherein the temperature in steps (a) and (b) is in the range from 0 to 40°C.

4. A process according to claim 1 wherein the temperature in steps (a) and (b) is about 70°C.

5. A process according to any one of claims 1 or 4 wherein the ratio of phosphorus to complex alcohols is substantially equimolar.

6. A process according to any one of claims 2 or 3 wherein the ratio of P_4O_{10} to complex alcohols is substantially equimolar.

7. A process according to claim 5 wherein the period of time in step (b) does not exceed about 30 minutes.

8. A process according to claim 6 wherein the period of time in step (b) does not exceed about 10 minutes.

9. A process according to any one of the preceding claims wherein the intimate mixture in step (a) is formed in the presence of an aliphatic acid.

10. A process according to claim 9 wherein the aliphatic acid is a free fatty acid.

11. A phosphate derivate of a complex alcohol which was produced according to any one of the above claims.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00452

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07F 9/11, 9/117, 9/12, 9/655

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07F 9/-

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT: C07F/- and (phosphorous pentoxide or polyphosphoric acid or phosphoric anhydride or diphosphorus pentoxide or phosphorus oxide or P4O10 or P2O5)
CHEMICAL ABSTRACTS: phosphorylation/ct and 1314-56-3/rn

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 141 938 (KLOSE) 27 February 1979 Examples	1-11
X	US 4 874 883 (UPHUES et al.) 17 October 1989 Examples	1-11
X	US 5 138 084 (CASAGRANDE et al.) 11 August 1992 Examples	1-11

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

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Date of the actual completion of the international search	Date of mailing of the international search report
14 July 2000	25 JUL 2000
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International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 554 781 (REIERSON) 10 September 1996 Examples	1-11
X	Derwent Abstract Accession no. 97-061803/06, Class B05 D21 E11, JP 08311085 (KAO CORP) 26 November 1996	1-11
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X	Patent Abstracts of Japan JP 10-045783 (SHOWA DENKO KK) 17 February 1998 abstract	1-11

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00452

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
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		FR	2367078	GB	1545193	IT	1091147
		NL	7711058	SE	7710788		
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		IT	1236843	JP	3176495		
US	5554781	CA	2145837	CN	1125729	EP	675076
		JP	8041082				

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